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# PERSONALISED THERAPY FOR MELANOMA

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**Summary**

In the therapy of metastatic melanoma, prior to 2011 the only approved treatment option were dacarbazine and interleukin-2, with a small percentage of patients with good response; no study involving these agents had shown an improvement in overall survival. Researches in the last decades have contributed to a better understanding of melanoma. The discovery that BRAF is a driver oncogene in cancer and complementary improvements in our understanding of the immune system have resulted in new targeted and immune-therapies for metastatic melanoma. Targeted therapies can achieve impressive clinical results in large number of patients, but the resistance to the therapy is often present. Immune therapy can achieve long-term remission and cures, yet in a smaller proportion of patients, and we still have no biomarkers to predict which patients will respond. Nevertheless, melanoma has led the evolution of cancer treatment from relatively non-specific cytotoxic agents to highly selective therapies, which offer an improvement in the outcome for melanoma patients. Still, many open questions remain: how to avoid resistance to therapy; how to find biomakers to predict answer to therapy; and how to find the optimal treatment options for patients who relapse or do not respond.

***Keywords***: metastatic melanoma; immunotherapy; targeted therapy.

## INTRODUCTION

Metastatic melanoma has a poor prognosis. The median survival for patients with stage IV melanoma ranging from 8 to 18 months after diagnosis,

depending on the substage. Few patients have a response to systemic therapies [1].

The only chemotherapeutic agent approved by the Food and Drug Administration for the treatment of metastatic melanoma for many years was dacarbazine. In phase 3 studies, dacarbazine was associated with a response rate of 7 to 12% and a median overall survival of 5.6 to 7.8 months after the initiation of treatment. Higher response rates can be achieved with combination chemotherapy, but these combinations have not resulted in improved rates of overall survival. Some other agents were used to treat metastatic melanoma- temozolomide, fotemustine, carboplatin, paclitaxel, and interleukin-2 and demonstrated limited efficacy, and no study involving these agents had shown an improvement in overall survival [1-5].

## BRAF INHIBITOR

The era of targeted therapy in melanoma began with the identification of driver mutations in the serine threonine kinase *BRAF*. Approximately 40-60% of melanomas harbor activating (V600) mutations in the serine–threonine protein kinase B-RAF (BRAF). Melanomas carrying a BRAF V600E mutation constitutively activate the mitogen-activated protein kinase (MAPK) pathway, promoting cell proliferation and preventing apoptosis [6].

**Vemurafenib** was developed as a potent kinase inhibitor with specificity for the BRAF V600E mutation within cancer cells. It has marked antitumor effects against melanoma cell lines with the BRAF V600E mutation but not against cells with wild-type BRAF [7].

The oral BRAF inhibitor vemurafenib frequently produced tumor regressions in patients with BRAF V600–mutant metastatic melanoma. A phase 1 trial established the maximum tolerated dose to be 960 mg twice daily and showed frequent tumor responses [8].

A phase 2 trial involving patients who had received previous treatment for melanoma with the BRAF V600E mutation showed a confirmed response rate of 53%, with a median duration of response of 6.7 months [9].

A phase 3 randomized clinical trial comparing vemurafenib with dacarbazine in 675 patients with previously untreated, metastatic melanoma with the BRAF V600E mutation. Patients were randomly assigned to receive either vemurafenib (960 mg orally twice daily) or dacarbazine (1000 mg per square meter of body-surface area intravenously every 3 weeks). At 6 months, overall survival was 84% (95% confidence interval [CI], 78 to 89) in the ve-

murafenib group and 64% (95% CI, 56 to 73) in the dacarbazine group. In the interim analysis for overall survival and final analysis for progression-free survival, vemurafenib was associated with a relative reduction of 63% in the risk of death and of 74% in the risk of either death or disease progression, as compared with dacarbazine (P<0.001 for both comparisons). Response rates were 48% for vemurafenib and 5% for dacarbazine. Benefit was seen in all subgroups of patients who were included in the analysis, including patients with stage M1c disease or an elevated lactate dehydrogenase level, both of which are associated with particularly poor prognoses [10].

Similar to the first selective serine/threonine-protein kinase B-raf inhibitor vemurafenib, another selective inhibitor **dabrafenib** is highly efficacious in melanoma patients with BRAF V600E mutations, with response rates of approximately 50% and progression-free survival of 6 months. There is data to suggest that dabrafenib not only shows activity in V600E-mutated melanoma, but also in non-V600E BRAF mutated disease such as V600K. Dabrafenib, an inhibitor of mutated BRAF, has clinical activity with a manageable safety profile in studies of phase 1 and 2 in patients with BRAF(V600) mutated metastatic melanoma. In fase 3 randomised controlled trial dabrafenib (187 patients) or dacarbazine (63 patients). Median progression-free survival was 5,1 months for dabrafenib and 2,7 months for dacarbazine, with a hazard ratio (HR) of 0,30 (95% CI 0·18-0·51; p<0·0001) [11].

## MEK INHIBITOR

As was already told, the most commonly observed BRAF mutation, V600E, and the next most common, V600K, account for 95% of the BRAF mutations found in all patients with cancer. Activated BRAF phosphorylates and activates MEK proteins (MEK1 and MEK2), which then activate downstream MAP kinases. The MAP kinase pathway is known to regulate proliferation and survival of tumor cells in many cancers.9 In preclinical models of human melanoma, selective BRAF and MEK inhibitors have inhibited growth and induced cell death in tumors bearing BRAF mutations [7].

**Trametinib** is an orally available, small-molecule, selective inhibitor of MEK1 and MEK2. In the phase 3 open-label trial, 322 patients who had metastatic melanoma with a V600E or V600K BRAF mutation were assigned to receive either trametinib, an oral selective MEK inhibitor, or chemotherapy in a 2:1 ratio. Patients received trametinib (2 mg orally) once daily or intravenous

dacarbazine (1000 mg per square meter of body-surface area) or paclitaxel (175 mg per square meter) every 3 weeks. Median progression-free survival was 4.8 months in the trametinib group and 1.5 months in the chemotherapy group (hazard ratio for disease progression or death in the trametinib group, 0.45; 95% confidence interval [CI], 0.33 to 0.63; P<0.001). At 6 months, the rate of overall survival was 81% in the trametinib group and 67% in the chemotherapy group despite crossover (hazard ratio for death, 0.54; 95% CI, 0.32 to 0.92; P=0.01). Rash, diarrhea, and peripheral edema were the most common toxic effects in the trametinib group and were managed with dose interruption and dose reduction; asymptomatic and reversible reduction in the cardiac ejection fraction and ocular toxic effects occurred infrequently. Secondary skin neoplasms were not observed. Trametinib, as compared with chemotherapy, improved rates of progression-free and overall survival among patients who had metastatic melanoma with a BRAF V600E or V600K mutation [12].

## BRAF + MEK INHIBITOR

As with most targeted therapies that block a driver oncogene, cancer cells can develop acquired resistance. Resistance to BRAF inhibitors in melanoma is complex and mediated through multiple mechanisms with heterogeneous patterns of progression observed. The currently available data have indicated that the MAPK pathway is reactivated in resistant tumors. Some initial investigations suggest that reactivation of the MAPK pathway through the emergence of truncated hyperactive forms of BRAF, [13] secondary mutations in NRAS (the neuroblastoma RAS viral oncogene homologue) [14] or MEK (MAP kinase kinase), [15] up-regulation of COT (also known as TPL2 or MAP3K8), [16] or activation of alternative survival pathways induced by in-creased expression of receptor tyrosine kinases but not by secondary point mutations in BRAF32,35 are all mechanisms of resistance [17].

*In vitro*, MAPK signalling recovers rapidly following BRAF inhibition, in part through the relief of feedback inhibition in the pathway and an increased sensitivity to growth factors such as epidermal growth factor (EGF), neuregulin (NRG-1), hepatocyte growth factor (HGF) and fibroblasts growth factor (FGF). [17] In this context, reactivation of MAPK signalling following BRAF inhibition is important for therapeutic escape with increased levels of cell death and tumour regression being seen when BRAF and MEK are co-targeted [18-19].

Clinical trials have confirmed these preclinical observations with the BRAF/MEK inhibitor combination (**dabrafenib plus trametinib**) showing an increased PFS compared with BRAF inhibitor alone. In open-label study (Flaherty 2012.)162 patients were randomly assigned to receive combination therapy with dabrafenib (150 mg) plus trametinib (1 or 2 mg) or dabrafenib monotherapy. The combination therapy 150/2 (full-dose) group had significantly longer progression-free survival than did the monotherapy group (hazard ratio, 0.39; 95% CI, 0.25 to 0.62; P<0.001). The percentage of patients who were alive and progression-free at 1 year was also substantially higher (41% vs. 9%, P<0.001). The extent of tumor regression was also greater in the combination 150/2 group, with an objective response rate of 76%, as compared with 54% with monotherapy (P = 0.03). In addition, the median duration of response was substantially improved with combination therapy, as compared with dabrafenib monotherapy (10.5 months vs. 5.6 months) [20].

Another BRAF/MEK inhibitor combination **vemurafenib+cobimetinib** also appears promising, with newly released data from the BRIM-7 trial demonstrating an 87% confirmed response rate by RECIST and a median PFS of 13.7 months [21].

In treating *BRAF*-mutant melanoma a lot of progress has been made in developing oncogene directed therapies. With BRAf inhibitor response has been reported in about 25% and 50% of patients, and the median duration of response is about 6–7 months.10 Combination therapy with BRAF and MEK inhibitors results in an objective response rate of 76% and extends progression-free survival (PFS), but most patients develop resistance to these inhibitors. [20]. The major problem that limits the long-term responsiveness of the patients is resistance to these drugs. Current strategies to improve the durability of response are now focused on the development of personalized combination therapy strategies, the majority of which point upon the suppression of adaptive MAPK and PI3K/AKT signaling. At this time, the relationship between the genetic prolife of the tumor and patterns of adaptive signaling are not well understood. Better assays and biomarkers will be needed to explore the early treatment responses. The analysis of circulating tumor cells, circulating tumor DNA and proteomic methods are the strategies that are exploring [22].

## CTLA-4 IMMUNOTHERAPY

Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) is an immune check-point molecule that down-regulates pathways of T-cell activation. **Ipilimumab**, is a fully human monoclonal antibody (IgG1) that blocks CTLA-4 on lymphocytes to promote antitumor immunity. [23] Ipilimumab has recently been associated with superior overall survival, with median overall survival of 10.1 months among previously treated patients and 11.2 months among previously untreated patients [24-25].

In the study leading to approval of ipilimumab, patients with unresectable stage III or IV melanoma, whose disease had progressed while they were receiving therapy for metastatic disease, were randomly assigned, in a 3:1:1 ratio, to receive ipilimumab plus gp100 (403 patients), ipilimumab alone (137), or gp100 alone (136). Ipilimumab, at a dose of 3 mg per kilogram of body weight, was administered with or without gp100 every 3 weeks for up to four treatments. The results showed median overall survival was 10.0 months among patients receiving ipilimumab plus gp100, as compared with 6.4 months among patients receiving gp100 alone (hazard ratio for death, 0.68; P<0.001). The median overall survival with ipilimumab alone was 10.1 months (hazard ratio for death in the comparison with gp100 alone, 0.66; P=0.003). No difference in overall survival was detected between the ipilimumab groups (hazard ratio with ipilimumab plus gp100, 1.04; P=0.76). Grade 3 or 4 immune-related adverse events occurred in 10 to 15% of patients treated with ipilimumab and in 3% treated with gp100 alone. There were 14 deaths related to the study drugs (2.1%), and 7 were associated with immune-related adverse events [24].

In the study with previously untreated metastatic melanoma, 502 patients were assigned to ipilimumab (10 mg per kilogram) plus dacarbazine (850 mg per square meter of body-surface area) or dacarbazine (850 mg per square meter) plus placebo, given at weeks 1, 4, 7, and 10, followed by dacarbazine alone every 3 weeks through week 22. Overall survival was significantly longer in the group receiving ipilimumab plus dacarbazine than in the group receiving dacarbazine plus placebo (11.2 months vs. 9.1 months, with higher survival rates in the ipilimumab-dacarbazine group at 1 year (47.3% vs. 36.3%), 2 years (28.5% vs. 17.9%), and 3 years (20.8% vs. 12.2%) (hazard ratio for death, 0.72; P<0.001). Grade 3 or 4 adverse events occurred in 56.3% of patients treated with ipilimumab plus dacarbazine, as compared

with 27.5% treated with dacarbazine and placebo (P<0.001). No drug-related deaths or gastrointestinal perforations occurred in the ipilimumab-dacarbazine group [25].

However, the majority of patients do not have a response to anti-CTLA4 antibody therapy and still need effective therapeutic options.

## ANTI PD-1

In patients with ipilimumab-refractory melanoma the distinct mechanism of action of anti-programmed-death-receptor-1 (PD-1) antibodies, might have activity [26].

PD-1 is expressed on antigen-stimulated T cells and induces downstream signalling that inhibits T-cell proliferation, cytokine release, and cytotoxicity. Melanoma and many other tumors suppress cytotoxic T-cell activity by expressing PD-1 ligand (PD-L1) on the cell surface. Anti-PD-1 and PD-L1 antibodies can reverse this T-cell suppression and induce long-lasting antitumour responses in patients with advanced solid tumors, including advanced melanoma [26].

**Pembrolizumab** (MK-3475, previously known as lambrolizumab) is a highly selective, humanised monoclonal IgG4-kappa isotype antibody against PD-1 that has shown strong clinical activity with an acceptable safety profile. In phase I trial pembrolizumab at a dose of 2 mg/kg or 10 mg/kg every 3 weeks might be an efective treatment in patients who progresed to ipilimumab [27].

## CONCLUSION

In melanoma we testify the evolution of cancer treatment from nonspecific cytotoxic agents to highly selective therapies. Recent advances in treatment of melanoma: the mitogen-activated protein kinase pathway inhibitors vemurafenib, dabrafenib, trametinib, anti-cytotoxic T-lymphocyte-associated-antigen-4 (CTLA-4) antibody ipilimumab, anti PD-1 pembrolizumab offer the patients improvements in outcomes. But still some challenges remain: how to avoid resistace to therapy, find the biomakers to predict answer to therapy and find the optimal treatment options for patients who relapse or do not respond.

## References

[1] Balch CM, Gershenwald JE, Soong S-J, et al. Final version of 2009 AJCC melano-ma staging and classification. J Clin Oncol. 2009;27:6199-206.

[2] Chapman PB, Einhorn LH, Meyers ML, et al. Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. J Clin Oncol. 1999;17:2745-51.

[3] Middleton MR, Grob JJ, Aaronson N, et al. Randomized phase III study of te-mozolomide versus dacarbazine in the treatment of patients with advanced met-astatic malignant melanoma. J Clin Oncol. 2000;18:158-66.6.

[4] Avril MF, Aamdal S, Grob JJ, et al. Fotemustine compared with dacarbazine in patients with disseminated malignant melanoma: a phase III study. J Clin Oncol. 2004;22:1118-25.

[5] Bedikian AY, Millward M, Peham-Berger H, et al. Bcl-2 antisense (oblimers-en sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. J Clin Oncol. 2006;24:4738-45.

[6] Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005;353:2135-47.

[7] Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949-54.

[8] Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010;363:809-19.

[9] Ribas A, Kim KB, Schuchter LM, et al. BRIM-2: an open-label, multicenter phase II study of vemurafenib in previously treat-ed patients with BRAFV600E mutation-positive melanoma. J Clin Oncol. 2011;29:Suppl:8509.

[10] Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AMM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA. BRIM-3 Study Group: Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N Engl J Med. 2011;364:2507-16.

[11] Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH Jr, Kaempgen E, Martín-Algarra S, Karaszewska B, Mauch C, Chiarion-Sileni V, Martin AM, Swann S, Haney P, Mirakhur B, Guckert ME, Goodman V, Chapman PB. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012;380(9839):358-65.

[12] Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, Dummer R, Trefzer U, Larkin JM, Utikal J, Dreno B, Nyakas M, Middleton MR, Becker JC, Casey M, Sherman LJ, Wu FS, Ouellet D, Martin AM, Patel K, Schadendorf D; METRIC Study Group: Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med. 2012;367(2):107-14. doi: 10.1056/NEJMoa1203421. Epub. 2012 Jun 4

[13] Poulikakos PI, Persaud Y, Janakira-man M, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature. 2011;480: 387-90.

[14] Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010;468:973-7.

[15] Wagle N, Emery C, Berger MF, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. J Clin Oncol. 2011;29:3085-96.

[16] Johannessen CM, Boehm JS, Kim SY, et al. COT drives resistance to RAF inhibi-tion through MAP kinase pathway reacti-vation. Nature. 2010;468:968-72.

[17] Villanueva J, Vultur A, Lee JT, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in mela-noma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell. 2010;18:683-95.

[18] Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, Huang A, Wong WL, Callahan MK, Merghoub T, Wolchok JD, de Stanchina E, Chandarlapaty S, Poulikakos PI, Fagin JA, Rosen N. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. *Cancer Cell*. 2012;**22**(5):668–82.

[19] Paraiso KH, Fedorenko IV, Cantini LP, Munko AC, Hall M, Sondak VK, Messina JL, Flaherty KT, Smalley KS. Recovery of phospho-ERK activity allows melanoma cells to escape from BRAF inhibitor therapy. *Br J Cancer. 2010;***102**(12):1724–30.

[20] Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA 3rd, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K, Weber J.: Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med. 2012;367(18):1694-703.

[21] Ribas A, Gonzalez R, Pavlick A, Hamid O, Gajewski TF, Daud A, Flaherty L, Logan T, Chmielowski B, Lewis K, Kee D, Boasberg P, Yin M, Chan I, Musib L, Choong N, Puzanov I, McArthur GA. Combination of vemurafenib and cobimetinib in patients with advanced BRAF-mutated melanoma: a phase 1b study. *Lancet Oncol. 2014;***15**(9): 954-65.

[22] Fedorenko IV, Gibney GT, Sondak VK, Smalley KS. Beyond BRAF: where next for melanoma therapy? See comment in PubMed Commons belowBr J Cancer. 2014; Sep 2. doi: 10.1038/bjc.2014.476.

[23] O'Day SJ, Hamid O, Urba WJ. Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): a novel strategy for the treatment of melanoma and other malignancies. Cancer. 2007;110:2614-27.

[24] Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711-23. [Erratum, N Engl J Med. 2010;363:1290.]

[25] Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for pre-viously untreated metastatic melanoma. N Engl J Med. 2011;364:2517-26.

[26] Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. Nat Immunol. 2013;14:1212–18

[27] Robert C, Hamid O, Ribas A, et al. Updated clinical eficacy and safety of MK-3475 (anti-PD-1 monoclonal antibody) in advanced melanoma. Pigment Cell Melanoma Res. 2013;26:993 (abstr).

Sažetak

## Personalizirano liječenje melanoma

U liječenju metastatskog melanoma prije 2011. godine, jedina dva odobrena lijeka bili su dakarbazin i interleukin 2., s malim postotkom bolesnika u kojih je zabilježen dobar odgovor. Niti jedna studija s tim lijekovima nije pokazala učinak na ukupno preživljenje. Istraživanja posljednjih desetljeća pridonijela su boljem razumijevanju melanoma. Otkriće BRAF mutacije kao važnog onkogena u melanoma i istovremeno razvijanje lijekova s djelovanjem na imunološki sustav, rezultirali su novim ciljanim i imunološkim terapijama za liječenje metastatskog melanoma. Ciljana terapija postiže impresivne kliničke rezultate u velikom broju bolesnika, ali se često razvija rezistencija na terapiju. Imunološka terapija postiže dugotrajnu remisiju, ali u manjem postotku pacijenata, a još uvijek nemamo biomarkere za predviđanje koji su to pacijenti koji će reagirati. Ipak, u terapiji melanom svjedočimo evoluciji u liječenju raka od relativno nespecifičnih citotoksičnih lijekova do vrlo selektivne terapije koja nudi poboljšanje u ishodu liječenja za pacijente s melanomom. Ipak, puno je još otvorenih pitanja: kako izbjeći pojavu rezistencije na liječenje, naći biomarkere kao prediktore dobrog odgovora na terapiju i utvrditi optimalan način liječenja za bolesnike nakon progresije i za one koji ne reagiraju na ove terapije.

***Ključne riječi:*** metastatski melanom; imunoterapija; ciljana terapija.

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